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Pathogenicity of two *Pratylenchus coffeae* populations from Brazil on coffee plants

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Summary – There is limited information on the influence of *Pratylenchus coffeae* on the growth and development of coffee plants, in spite of the widespread occurrence of this nematode in coffee plantations. In addition, populations of *P. coffeae* vary in morphological and molecular features, as well as reproductive fitness and pathological potential. The objective of the present study was to compare the pathogenicity of two Brazilian *P. coffeae* populations, K_5 from *Coffea arabica* roots and M_2 from *Aglaonema* sp. roots, in terms of their influence on the plant growth and photosynthesis of Arabian coffee (*C. arabica*). Five experiments were conducted in controlled conditions, and the results demonstrated that K_5 is pathogenic on coffee, as it can reproduce and causes obvious damage on the plant. Moreover K_5 proved to be very virulent on Arabian coffee, considering its effects on seedling mortality, plant growth and photosynthesis. By contrast, M2 was considered to be of low virulence, or even non-pathogenic, on coffee because it failed to reproduce. Thus, the results indicate that K_5 and M_2 may be distinct species, supporting the hypothesis of previous authors.

Keywords – *Coffea arabica*, diversity, pathogenicity, photosynthesis, plant growth, root-lesion nematode.

Three species of root-lesion nematodes are parasitic on coffee (*Coffea arabica* L. and *C. canephora* Pierre) in Brazil: *Pratylenchus brachyurus* (Godfrey) Filipjev & S. Stekhoven, *P. coffeae* (Zimmermann) Filipjev & S. Stekhoven and *P. vulnus* Allen & Jensen (Kubo *et al.*, 2004). Although *P. coffeae* is widespread and a major coffee pest in a number of countries in the Caribbean, Central America, Africa and Asia (Schieber & Grullon, 1969; Campos *et al.*, 1990; Kumar & Samuel, 1990), there are only limited studies of its effects on coffee under controlled conditions.

In Costa Rican coffee plantations, Salas and Echandi (1961) related low harvest, leaf wilt, chlorosis and shedding to poor root development in adult plants infected with *P. coffeae*. Similar symptoms were described in Brazilian coffee plantations infested with the same nematode (Monteiro & Lordello, 1974). According to Kumar (1982), *P. coffeae* enters the roots of coffee plants after rupturing the epidermis. During its migration through epidermal and cortical cells, *P. coffeae* breaks down cell walls and provokes enlargement of cells, resulting in a slight swelling of the root surface.

Host-range variability in *P. coffeae* has been observed in Central America since 1973 (Edwards & Wehunt, 1973). Currently, *P. coffeae* is the subject of taxonomic debate because recent studies have shown important morphological and molecular differences among its populations (Duncan *et al.*, 1999; Wilcken *et al.*, 2002a, b). Two Brazilian populations of *P. coffeae*, namely K_5 from Arabian coffee *C. arabica* cv. Mundo Novo collected in Marília and M_2 from *Aglaonema* sp. collected in Rio de Janeiro, can be differentiated by molecular and morphometric features and by their host-range (Duncan *et al.*, 1999; Silva & Inomoto, 2002).

According to Silva and Inomoto (2002), K_5 reproduced very slowly and M_2 failed to reproduce on *C. arabica*, suggesting that coffee is a poor host for K_5 and a non-host for M_2. However, *P. coffeae* has been found causing damage in Brazilian coffee plantations. For example, Kubo *et al.* (2004) reported that *P. coffeae* occurred in

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5.1% of coffee roots sampled in São Paulo state (average of 94 nematodes per g root). These contradictory results may be due to the low reproduction rate of *P. coffeae* on coffee; thus, many months or years are needed before *P. coffeae* reaches high densities in coffee roots. As coffee is a perennial crop, such high densities are possible in commercial plantations, resulting in the symptoms reported by Salas and Echandi (1961), Monteiro and Lordello (1974) and Kumar (1982).

Therefore, studies regarding the reproductive fitness and virulence of the different populations of *P. coffeae* on coffee should be more thoroughly investigated in long-term experiments using low and high densities of the nematode. There are conflicting concepts of pathogenicity, reproductive fitness and virulence (Shaner *et al.*, 1992). In the present work, pathogenicity is defined as the capacity of an organism, like the nematode, to cause disease on the plant host, reproductive fitness is defined as the ability of the nematode to reproduce on the host, and virulence is defined as the amount of damage caused by the nematode on the host. The objectives of this work were to study the pathogenicity and the reproductive fitness of two Brazilian *P. coffeae* populations, K_5 and M_2, on Arabian coffee plants, and to compare the virulence of these populations on coffee.

Materials and methods

Five experiments were conducted under controlled glasshouse conditions (soil temperature ranged from 15 to 28°C) at the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Piracicaba, SP, Brazil (22°43′9″S and 47°36′59″W).

High initial densities of K_5 and M_2 were used in two long-term trials (Experiments 1 and 2), in order to confirm the pathogenicity of these nematodes on coffee, to compare the virulence of populations K_5 and M_2, and to achieve, if possible, symptoms similar to those reported by Salas and Echandi (1961), Monteiro and Lordello (1974) and Kumar (1982). There was marked damage caused by K_5 in Experiments 1 and 2, so lower initial densities of K_5 and M_2 were used in Experiments 3 and 4, in order to evaluate the reproductive fitness of both populations on coffee.

General procedures

Previously, the populations of *P. coffeae* used in this study were characterised morphologically and by host-range by Duncan *et al.* (1999) and Silva and Inomoto (2002); K_5 were from *C. arabica* roots and M_2 were from *Aglaonema* sp. roots. Both populations were maintained on alfalfa callus (Riedel *et al.*, 1973) by periodic subculturing and their inocula were obtained from callus 45 to 90 days after infection using a modified Baermann method (Hooper, 1986).

Seedlings of the two most cultivated genotypes of coffee in Brazil (*C. arabica* cvs Mundo Novo and Catuaí) were obtained from seeds germinated in moist sand. Two months after sowing, seedlings with two expanded cotyledon leaves were transferred to 500 cm^3 plastic pots (one seedling per pot) containing 450 cm^3 of substrate (18% clay, 6% silt, 76% sand; 1% organic material and pH 5.9) fumigated with methyl bromide (150 cm^3 of CH_3Br per m^3 of substrate). One or 2 months after transplanting the seedlings, when they had one or two pair of leaves, the substrate of the pots was inoculated with an aqueous suspension containing mixed stages of *P. coffeae*, at the required concentration (see below) for the initial population (*Pi*). The inoculum was distributed into two holes of 2 cm depth situated at 1 cm from either side of the stem of the coffee seedling. At the end of the experimental period, nematode final population (*Pf*) was estimated by counting nematodes extracted from roots (Coolen & D'Herde, 1972) and substrate (Jenkins, 1964). Then, nematode number per g roots (nem/g) and nematode reproduction (*Pf*/*Pi*) was calculated for each replicate.

Photosynthesis was evaluated by measuring the chlorophyll fluorescence under non-saturated light, using a Photosynthesis Yield Analyzer MINI-PAN (Heinz Walz, Effeltrich, Germany). Photosynthesis measurements were taken on completely expanded leaves under full sunlight, between 9:50 and 10:10 am (light radiation between 900 and 1000 mol m^{-2} s^{-1}). During the measurement period, the light radiation was almost stable; thus, the yield parameter obtained from this equipment reflected the general photosynthetic efficiency.

Experiments 1 and 2 – effects of high population densities of K_5 and M_2 on photosynthesis and growth of *Coffea arabica* cvs Mundo Novo and Catuaí

Seedlings of cv. Mundo Novo with two pairs of leaves received three treatments: control (non-infected plants), nematodes from K_5 population and nematodes from M_2 population. Aqueous suspensions containing 8000 juveniles and adults of K_5 or M_2 (17.8 nematodes per cm^3

of substrate) were poured into the two 2 cm holes either side of the plant stem.

Coffee seedlings were maintained in a shaded room for 12 h to avoid heat stress on the nematodes, before being transferred to the glasshouse. Nutrient solution ($N:P_2O_5:K_2O:Ca:Mg:S$, ratio 15:15:20:1:4:0.4) was used once, 140 days after inoculation (dai), when symptoms of nutritional deficiency were visually observed. After 158 dai, the plants were transferred to 2100 cm^3 pots containing 1300 cm^3 of fumigated substrate. At the end of the experimental period (261 dai), the effect of K_5 and M_2 on photosynthesis was evaluated by measuring the yield parameter, and the effect on coffee growth by measuring the length of coffee stem from substrate level to the terminal bud shoot (plant height). The coffee stem was then cut at substrate level and oven dried at 70°C for 3 days in order to obtain the shoot dry weight. Root fresh weight was also measured and the final population (*Pf*) of both *P. coffeae* populations on coffee cv. Mundo Novo was calculated by counting the nematodes extracted from roots and substrate.

Experiment 2 was similar to Experiment 1, but extending for only 201 dai and used the coffee cv. Catuaí.

Experiments 3 and 4 – effects of two densities of K_5 on photosynthesis and growth of *Coffea arabica* cvs Mundo Novo and Catuaí

In Experiment 3, inoculation of K_5 was carried out on seedlings of cv. Mundo Novo with one pair of leaves. The treatments were three initial densities (*Pi*) of the K_5 population: 0 (uninfected control); 1000 nematodes per pot (2.2 nematodes per cm^3 of substrate); and 3000 nematodes per pot (6.7 nematodes per cm^3 of substrate). In order to maintain the coffee plants in good conditions, avoiding nutritional deficiency symptoms, they received a nutrient solution ($N:P_2O_5:K_2O:Ca:Mg:S$, ratio 15:15:20:1:4:0.4) at 159, 187, 202 and 221 dai. Photosynthesis was evaluated 208 and 260 dai, and plant height was determined at 188 and 260 dai. At the final evaluation (260 dai), shoot dry weight, root fresh weight, and nematode final population (*Pf*) were also determined. Experiment 4 was similar to Experiment 3 and conducted concurrently, but using the coffee cv. Catuaí.

Experiment 5 – effects of two densities of M_2 on growth of *Coffea arabica* cv. Catuaí

Seedlings of cv. Catuaí with two pairs of leaves received three treatments, corresponding to the initial densities (*Pi*) of the M_2 population of *P. coffeae*: 0 (uninfected control); 1000 nematodes per pot (2.2 nematodes per cm^3 of substrate); and 3000 nematodes per pot (6.7 nematodes per cm^3 of substrate). The evaluation was made 147 dai by measuring the plant height, shoot dry weight, root fresh weight and nematode final population (*Pf*). Photosynthesis was not measured in this experiment because results from Experiments 1 and 2 showed that even at the higher density of M_2 ($Pi = 8000$ nematodes per plant or 17.8 per cm^3) the photosynthesis of coffee plants was not affected.

Data analysis

The experiments were set in completely randomised design with 12 (Experiments 1, 2, 3 and 4) or eight replicates (Experiment 5). Data of growth (plant height, shoot dry weight, and root fresh weight) and photosynthesis did not need normalisation and were analysed using SANEST software (Departamento de Matemática e Estatística, ESALQ/USP, Piracicaba, Brazil). Means were compared by Tukey's honestly significant difference test.

Data of nematodes per g fresh roots and reproduction (Pf/Pi) of K_5 and M_2 were tested and compared by rank-sum test (Wilcoxon's test) (Campos, 1983).

Results

Coffee seedlings infected with the K_5 population of *P. coffeae* showed leaf chlorosis and leaf shedding in Experiments 1 and 2, in spite of nutrient supplementation. Individual lesions among white and healthy tissues were not observed but most of the secondary and tertiary roots were dark brown and rotted; the distal part of the main root was decayed. Seedlings infected with M_2 did not exhibit leaf chlorosis or shedding. All roots were light brown but decay of tissues was not evident. From 12 replicates of coffee in Experiments 1 and 2, K_5 caused more seedling mortality than M_2: four *vs* one on cv. Mundo Novo, and two *vs* zero on cv. Catuaí. No control plants died in either experiment.

Photosynthesis was not affected by either population K_5 or M_2 in Experiment 1 (cv. Mundo Novo), and only by K_5 in Experiment 2 (cv. Catuaí). Both *P. coffeae* populations depressed the growth of coffee plants but greater adverse effects on plant height, shoot dry weight and root fresh weight were associated with K_5 rather than with M_2. Coffee supported more nematodes per g root of K_5 than M_2, but total numbers of nematodes decreased for both populations (Table 1).

Table 1. *Effect of 8000 nematodes of K_5 and M_2 populations of* Pratylenchus coffeae *on plant survival, photosynthesis and growth (plant height, shoot dry weight, and root fresh weight) of* Coffea arabica *cvs Mundo Novo (261 dai) and Catuaí (201 dai); values for nematodes per g roots, and nematode reproduction are also given.*

	cv. Mundo Novo			cv. Catuaí		
Pi	0 (control)	8000 M_2	8000 K_5	0 (control)	8000 M_2	8000 K_5
N	0	1	4	0	0	2
Phot*	0.1296 a	0.1018 a	0.1023 a	0.1201 a	0.0965 a	0.0397 b
Pl ht*	40.9 a	22.9 b	11.0 c	26.3 a	20.1 b	10.5 c
St wt*	10.5 a	2.9 b	0.7 c	8.4 a	5.7 b	1.1 c
Rt wt*	27.4 a	9.0 b	2.1 c	27.0 a	15.8 b	2.2 c
Nem/g**	–	61 b	1250 a	–	273 b	2384 a
*Pf/Pi***	–	0.06 b	0.43 a	–	0.50 a	0.25 a

Abbreviations: N, number of dead plants at the end of the experiment; Phot, photosynthesis yield; Pl ht, plant height (cm); St wt, shoot dry weight (g); Rt wt, root fresh weight (g); Nem/g, nematodes per g of fresh root; *Pf/Pi*, nematode reproduction.

* Means followed by the same letter in the row for the same coffee cultivar did not differ according to Tukey's test at 0.05 level.

** Means followed by the same letter in the row for the same coffee cultivar did not differ according to rank sum test (Wilcoxon's test) at 0.02 level.

It was observed that coffee plants infected with 1000 and 3000 nematodes of K_5 population in Experiments 3 and 4 had a similar appearance to plants infected with 8000 nematodes of K_5 in Experiments 1 and 2. From 12 replicates of cv. Mundo Novo, two infected with 1000 K_5 nematodes and eight with 3000 died during Experiment 3; the seedling mortality was equivalent in replicates of cv. Catuaí in Experiment 4: four ($Pi = 1000$) and five ($Pi = 3000$). No control plants died.

Plant responses to K_5 infection were similar on cvs Mundo Novo and Catuaí. The nematode depressed photosynthesis only at the $Pi = 3000$ and at the final evaluation (260 dai). However, growth of coffee plants was depressed at the two *Pi* levels, which did not differ from each other. The effect of K_5 on coffee growth was significant in the first evaluation (188 dai; stem height) and was maintained in the final evaluation (260 dai; stem height, shoot dry weight and root fresh weight). Lower numbers of nematodes per g roots and total number of nematodes were recovered from plants with high *Pi* (3000) than from plants with low *Pi* (1000). In fact, K_5 numbers increased at $Pi = 1000$ but decreased at $Pi = 3000$ (Table 2). The M_2 population did not cause plant mortality during Experiment 5. The growth of cv. Catuaí was depressed by M_2 at both *Pi* levels (Table 3). Contrary to Experiments 3 and 4, greater numbers of nematodes per g roots were recovered from plants with high *Pi* (1000) than from plants with low *Pi* (3000), but the total number of nematodes decreased for both *Pi* (Table 2).

Discussion

The extensive root destruction observed in coffee plants infected with the K_5 population of *P. coffeae* demonstrated that it is pathogenic to coffee. Probably, the damage caused by the K_5 population in coffee roots is the main factor that explains the other symptoms observed, *i.e.*, leaf shedding, low photosynthesis, reduced plant growth and high plant mortality observed in coffee plants infected with K_5. The findings concerning effects of K_5 population on coffee photosynthesis are in agreement with Mazzafera *et al.* (2004), who showed that inhibition of photosynthesis and sucrose transport from leaves to roots was indirectly caused by K_5, as that population can destroy the roots. Another previous report demonstrated that K_5 is a very virulent pathogen on *C. arabica*, causing damage in plant growth comparable to *Meloidogyne incognita* at $Pi = 2000$ in two glasshouse experiments (Inomoto *et al.*, 2004).

At high values of *Pi* (8000 in Experiments 1 and 2, and 3000 in Experiments 3 and 4), the effect of K_5 resulted in such intense tissue decay, after 200-260 dai, that the roots were unsuitable for nematode colonisation, thus explaining the decrease in numbers of K_5 nematodes in roots at *Pi* of 3000 in Experiments 3 and 4. At such high *Pi*, the *Pf/Pi* was lower than 1.0, suggesting incorrectly that coffee is a non-host for K_5. However, the numbers of K_5 increased at *Pi* of 1000, demonstrating that it is certainly able to reproduce in coffee roots. Therefore, the pathogenicity of K_5 to coffee is directly related to the

Table 2. *Effect of two densities (*Pi*) of the K_5 population of* Pratylenchus coffeae *on plant survival, photosynthesis and growth (plant height, shoot dry weight and root fresh weight) of* Coffea arabica *cvs Mundo Novo and Catuaí; values for nematodes per g roots, and nematode reproduction are also given.*

	cv. Mundo Novo			cv. Catuaí		
Pi	0	1000	3000	0	1000	3000
N 260 dai	0	2	8	0	4	5
Phot 208 dai*	0.1002 a	0.0858 a	0.0740 a	0.0945 a	0.0845 a	0.0569 a
Phot 260 dai*	0.1028 a	0.0746 ab	0.02450 b	0.1452 a	0.0961 ab	0.0577 b
Pl ht 188 dai*	16.9 a	7.5 b	6.9 b	12.0 a	6.4 b	5.8 b
Pl ht 260 dai*	30.1 a	8.5 b	8.1 b	19.5 a	7.7 b	6.6 b
Sh wt 260 dai*	4.54 a	0.22 b	0.09 b	4.45 a	0.47 b	0.15 b
Rt wt 260 dai*	10.7 a	0.7 b	0.5 b	12.7 a	1.8 b	0.6 b
Nem/g 260 dai**	–	414 a	202 b	–	1271 a	327 b
Pf/*Pi* 260 dai**	–	1.16 a	0.33 b	–	4.33 a	0.19 b

Abbreviations: N, number of dead plants at the end of the experiment; dai, days after inoculation; Phot, photosynthesis yield; Pl ht, plant height (cm); St wt, shoot dry weight (g); Rt wt, root fresh weight (g); Nem/g, nematodes per g of fresh root; *Pf*/*Pi*, nematode reproduction.

* Means followed by the same letter in the row for the same coffee cultivar did not differ according to Tukey's test at 0.05 level.

** Means followed by the same letter in the row for the same coffee cultivar did not differ according to rank sum test (Wilcoxon's test) at 0.02 level.

Table 3. *Effect of two densities (*Pi*) of the M_2 population of* Pratylenchus coffeae *on plant survival, growth (plant height, shoot dry weight and root fresh weight) of* Coffea arabica *cv. Catuaí; values for nematodes per g roots, and nematode reproduction after 147 days are also given.*

	Pi		
	0	1000	3000
N	0	0	0
Pl ht*	11.1 a	9.4 ab	8.7 b
Sh wt*	1.36 a	0.86 b	0.80 b
Rt wt*	1.99 a	1.06 b	1.02 b
Nem/g**	–	375 b	1082 a
Pf/*Pi***	–	0.44 a	0.41 a

Abbreviations: N, number of dead plants at the end of the experiment; Pl ht, plant height (cm); St wt, shoot dry weight (g); Rt wt, root fresh weight (g); Nem/g, nematodes per g of fresh root; *Pf*/*Pi*, nematode reproduction.

* Means followed by the same letter in the row for the same coffee cultivar did not differ according to Tukey's test at 0.05 level.

** Means followed by the same letter in the row for the same coffee cultivar did not differ according to rank sum test (Wilcoxon's test) at 0.02 level.

capacity of the nematode to colonise and reproduce in coffee roots.

Compared with K_5, the effect of M_2 on coffee roots was less pronounced. Coffee plants infected with the population M_2 of *P. coffeae* were more developed than with K_5, and appeared healthier, without leaf shedding. Only one plant with M_2 died in three experiments (1, 2, 5). Numbers of M_2 decreased at all *Pi* used (8000 in Experiments 1 and 2, and 1000 and 3000 in Experiment 5), results which are in agreement with those of Silva and Inomoto (2002) and corroborate the finding that coffee is a better host for K_5 than for M_2, or even that coffee is non-host for M_2. As nematode invasion occurred for both K_5 and M_2 populations, we hypothesised that the main difference between K_5 and M_2 is the capacity to reproduce in coffee roots. As M_2 failed to colonise and reproduce on coffee, the damage caused by M_2 is a direct consequence of the process of nematode root penetration. Therefore, this population is probably a low virulent pathogen or even non pathogenic on coffee. The presence of populations of *P. coffeae* in Brazil with low reproductive fitness, *e.g.*, M_2 and, correspondently, low virulence on coffee, might explain the restricted geographical distribution of *P. coffeae* in Brazilian coffee plantations, as reported by D'Antonio *et al.* (1980) and Kubo *et al.* (2004).

Therefore, the present results demonstrated that both *P. coffeae* populations (K_5 and M_2) can depress the growth of Arabian coffee plants. However, only K_5 can reproduce

and is undoubtedly pathogenic on coffee. Since K_5 and M_2 can be differentiated by molecular and morphometric features (Duncan *et al.*, 1999; Wilcken *et al.*, 2002a, b) and by their host-range (Silva & Inomoto, 2002), the present results, concerning reproductive fitness and virulence on coffee, add more weight to the hypothesis that K_5 and M_2 are distinct species. As the development of management strategies for K_5 and M_2 will depend on the definition of the correct taxonomic status of these populations, future research should investigate this aspect.

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